*Submission type: Research article*

**Generalist predators function as pest specialists: examining diet composition of spiders and ladybeetles across rice crop stages**

**Abstract**

1. Biocontrol, using natural enemies for pest control, has a long history in agriculture. To solve a long-standing puzzle in biocontrol—how well the ubiquitous generalist arthropod predators (GAPs) function as biocontrol agents—this study aimed to 1) quantify the diet composition of GAPs (spiders and ladybeetles) at different crop stages using stable isotope analysis, 2) examine the consistency of GAPs in pest consumption over years, and 3) investigate how various factors such as farming practice, surrounding vegetation, and prey abundance affect pest consumption by GAPs.
2. We sampled arthropod prey and GAPs in seven pairs of subtropical organic and conventional rice farms at major rice growth stages (tillering, flowering, and ripening) for three consecutive years (2017-2019). Among our sweep-net samples, we analyzed 352 predator and 828 prey isotope samples to infer predator-prey interactions.
3. Our results show the following: a) The proportion of rice pests in GAPs’ diets in both organic and conventional rice farms increased over the crop season, from 21-47% at the tillering stage to 76-97% at the flowering and ripening stage, across the three study years. The high pest consumption at late crop stages (flowering and ripening stage) suggests that GAPs can function as pest specialists during the critical period of crop production. Regarding individual predator groups, spiders and ladybeetles exhibited distinct dietary patterns over the crop season. b) The high pest consumption by GAPs at late crop stages was similar across years despite variable climatic conditions and prey availability, suggesting a consistency in GAPs’ feeding habits and biocontrol value. c) The proportion of rice pests in GAPs’ diets varied with farm type and crop stage (e.g., higher in conventional farms and during flowering/ripening stages).
4. By quantifying the diet composition of GAPs over the growing season in different farm types across years, this study reveals that generalist predators have potential to produce a stable, predictable top-down effect on pests in rice agro-ecosystems. Therefore, promoting the densities of ubiquitous generalist arthropod predators in the field will likely benefit pest management and help achieve sustainable agriculture.

*Keywords: biocontrol, trophic interactions, generalist predators, rice paddy, organic and conventional farms, stable isotope analysis*

**1. Introduction**

Using natural arthropod enemies for pest control has a long history in agriculture. The earliest record of biocontrol was documented in the book *Plants of the Southern Regions* (*ca.* 304 A.D.): people sold ants and their nests in the markets to control citrus insect pests (Huang and Yang, 1987). While synthetic pesticides have become the main method for controlling pests in the past century, this comes at a cost, such as posing risks to people, reducing biodiversity and hampering ecosystem functions (Geiger *et al.*, 2010; Kehoe *et al.*, 2017). As agriculture has become the largest land use type worldwide and a major driver for the global biodiversity crisis in Anthropocene (Campbell *et al.*, 2017), a shift from synthetic pesticides to environmentally friendly practices (e.g., biocontrol) is urgently needed to make agriculture more sustainable (Gomiero *et al.*, 2011). For example, the European Commission has announced its plan to reduce the use of chemical pesticides in European Union agricultural systems by 50% by 2030 (European Commission, 2020). To achieve this ambitious sustainability goal, biocontrol by natural enemies has been considered a key approach and has regained importance in modern agriculture.

Natural enemies used for pest control can be classified into two major groups based on their prey range: specialist and generalist predators. While specialist predators (e.g., parasitoid wasps) have been widely advocated in agriculture because they target specific pest species and produce less undesirable non-target effects (Stiling and Cornelissen, 2005), generalist predators, despite their potential interference with each other (e.g., intraguild predation and cannibalism in spiders [Cuff *et al.*, 2021]), have been increasingly appreciated for their conspicuous existence and consistent biocontrol effect on pests (Symondson *et al.*, 2002; Stiling and Cornelissen, 2005; Michalko *et al.*, 2019; Mezőfi *et al*., 2020; Hsu *et al.*, 2021; Morente and Ruano, 2022; Gajski *et al.*, 2023). For example, generalist predators are common in various agro-ecosystems and have been reported to significantly reduce pest abundance in approximately 75% of 181 field manipulative studies (Symondson *et al.*, 2002). Moreover, a meta-analysis suggests that generalist predators may exert stronger biocontrol effects on pest populations over time compared to specialists (Stiling and Cornelissen, 2005).

While the value of generalist predators has been increasingly appreciated, a few fundamental knowledge gaps regarding predator-pest trophic interactions need to be filled to better understand their role as biocontrol agents in agro-ecosystems. For example, while studies have qualitatively analyzed the diets of generalist predators (e.g., using molecular gut content analysis to identify prey species) (Eitzinger and Traugott, 2011; Ingrao *et al.*, 2017; Albertini *et al.*, 2018), very few have quantified their diet composition over a growing season in the field (knowledge gap 1) (Hsu *et al.*, 2021; Otieno *et al.*, 2023). Besides the ability of generalist predators to suppress pest populations, it is also important to quantify their diet composition to address the concern that generalist predators may switch their diet from focal pests (which have large direct impacts on crop performance) to alternative prey (which have minor direct impacts on crop performance), thereby reducing pest control effectiveness (Michalko *et al.*, 2019). For instance, if generalist predators consume a high proportion of pests in their diets even in the presence of alternative prey in the field, this would help end a long debate on whether generalist predators serve well as biocontrol agents (Symondson *et al.*, 2002; Krey *et al.*, 2017; Michalko *et al.*, 2019). Moreover, fluctuations in abiotic factors and habitat conditions can lead to seasonal and yearly variation in prey population densities and species compositions in agro-ecosystems (Settle *et al.*, 1996; Wardle *et al*., 1999; Dominik *et al.*, 2018), which can in turn influence the foraging behavior of predators. Therefore, examining the consistency in pest consumption by generalist predators in the field over years will provide strong support for applying these predators in pest management programs as stable biocontrol agents, yet this information is currently lacking (knowledge gap 2).

To understand the mechanisms underlying the biocontrol effects of generalist predators, we also need to examine how various factors affect the diet composition of generalist predators in agro-ecosystems (knowledge gap 3). First, foraging behavior of generalist predators is largely governed by prey availability and predator-predator interactions (e.g., intraguild predation). Because arthropod communities (e.g., predator and prey richness/densities) can vary substantially throughout the crop growing season, it is important to examine how pest consumption by generalist predators changes over crop stages (Roubinet *et al.*, 2017). Second, farming practices (e.g., organic and conventional practice) can alter predator and prey communities in the field, thereby influencing the diet composition of predators (Birkhofer *et al.*, 2011). Therefore, examining pest consumption by predators in organic and conventional farms will help evaluate whether generalist predators can provide stable biocontrol services in different farm types. Third, generalist predators may exhibit preference over certain prey items, and investigating the relationship between the relative prey abundance in the field and the proportions of different prey items consumed in predators’ diets will help clarify whether pest availability or predator preference drives pest consumption by predators (Wise *et al.*, 2006; Kuusk and Ekbom, 2012; Roubinet *et al.*, 2017; Eitzinger *et al.*, 2019). Lastly, surrounding vegetation can affect arthropod diversity and predator-prey interactions in agro-ecosystems (Altieri and Letourneau, 1982; Altieri, 1999; Barbosa and Castellanos, 2005; Diehl *et al.*, 2013; Lichtenberg *et al.*, 2017). However, its effect on predators’ diets is unclear. Understanding this will provide insights for managing the agricultural landscape and promoting biocontrol services by generalist predators.

To address these three knowledge gaps, this study aimed to 1) quantify the diet composition of generalist predators, 2) examine the consistency of predators in pest consumption over years, and 3) investigate how various factors such as farming practice, surrounding vegetation, and prey abundance may affect the pest consumption by these predators. Filling these gaps will provide insights for applying generalist predators in biocontrol programs. Specifically, we sampled arthropod prey and generalist arthropod predators (GAPs) in sub-tropical organic and conventional rice farms over the rice growth season (seedling, tillering, flowering, and ripening stages) in central Taiwan from 2017 to 2019, and quantified the diet composition of GAPs (ladybeetles and spiders) at each rice stage using stable isotope analysis (δ13C and δ15N). Stable isotope analysis has been widely applied in trophic ecology to estimate the proportional contribution of different prey sources to the total diets of predator individuals within a species or a trophic group (Post, 2002; Boecklen *et al.*, 2011; Layman *et al.*, 2012). Compared to “snap-shot” techniques (e.g., field observations and molecular gut content analysis) that provide mainly qualitative information on the presence/absence of prey items in predators’ diets, stable isotope analysis can quantify the proportions of different prey items in predators’ diets integrated over a time period, allowing for better inferences about predator-prey interactions (Newton, 2016). Although GAPs may consume various prey items, we expected that GAPs would consistently consume a high proportion of pests in their diets at late crop stages (with high pest densities) regardless of years. We also expected farming practice, surrounding vegetation (percent forest cover), and the relative abundance of prey to affect pest consumption by GAPs.

**2. Materials and Methods**

*2.1. Study system and sample collection*

We collected terrestrial arthropods in paired organic and conventional rice farms in subtropical Taiwan (120.656-120.721 °E; 24.364-24.489 °N) from 2017 to 2019 (three farm pairs in 2017 and seven farm pairs each in 2018 and 2019) (Fig. Aa). While farms in the same pair were relatively close to each other (e.g., within a few hundred meters in distance), different farm pairs were at least 1 km apart from each other to reduce confounding effects. The study farms were 0.2 hectares on average and irrigated with surface water. The organic farms were managed with organic fertilizers (manure; 2-3 applications/crop season) and natural pesticides (tea saponins; 1 application/crop season during the seedling or tillering stage). The conventional farms were managed with synthetic nitrogen fertilizers (2-3 applications/crop season) and organophosphate pesticides (1 application/crop season during the tillering or flowering stage). At each major rice crop stage (seedling, tillering, flowering, and ripening stages) during the growing season (April - July) in each study year, we collected arthropod samples by sweep-netting (36 cm in diameter with a mesh size of 0.2 × 0.2 mm) the crop canopy 30 times in each of two transects inside a rice field (Fig. Ab). Each transect (ca 30 m long) was parallel to but 1.5 m away from a randomly selected farm ridge. Samples from the two transects were pooled and sealed in bags without chemical preservatives, iced, and transferred to refrigerator (−20ºC) in the laboratory. We identified and counted arthropods under a dissecting scope to the lowest possible taxonomic level (usually species, genus, or family). Main orders, families, and genera have been documented in a previous study by Hsu et al. (Hsu *et al.*, 2021) (note that the samples in year 2018 in this study were the same as those in Hsu *et al*. [2021]).

*2.2. Stable isotope analysis of arthropod samples*

After identification, arthropod samples were prepared for stable isotope analysis. First, samples were oven dried (50ºC) for one week, ground, and weighed into individual tin capsules (5 × 9 mm). If necessary, several conspecifics would be pooled into a capsule to meet the minimum weight required for stable isotope analysis (i.e., 2 mg in this study). The number of isotope capsules for each species generally mirrored the arthropod community composition in the field. Stable isotope analysis (352 arthropod predator and 828 prey isotope samples) was conducted at the UC Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The standards for carbon and nitrogen stable isotope ratios were Vienna PeeDee Beleminte and atmospheric N2, respectively. The results of our samples were expressed in per mil (‰) relative to the international standards (δ13C and δ15N).

*2.3. Arthropod trophic guild assignment*

A trophic guild represents a group of species using similar resources and forms a basic component of food webs. The concept has been proved to be practical in current ecology because it condenses broad taxonomic information into distinct functional groups in communities (Blondel, 2003). In this study, we classified arthropod samples into four trophic guilds (one predator and three prey guilds): 1) “Predators” consisted of spiders and ladybeetles, which are the primary GAPs in rice farms. 2) “Rice herbivores” consisted of major rice pests, including planthoppers, leafhoppers, and stink bugs. 3) “Tourist herbivores” consisted of herbivorous species without direct trophic association with rice plants, including some grasshoppers and leaf beetles. 4) “Detritivores” consisted of arthropods that feed on decaying organic material or plankton, including various midge and fly species. The classification of prey guilds was based on a combination of literature surveys and k-means clustering of stable isotope signatures of arthropod samples (see Appendix A: Fig. S1 for a stable isotope biplot for the three prey sources). The arthropod families/genera in each trophic guild are detailed in Appendix A: Table S1. This study focused on the trophic interactions between generalist predators and their prey sources and therefore did not consider less abundant trophic guilds (e.g., parasitoids) in subsequent analyses.

*2.4. Data analyses*

To quantify the diet composition of predators, we constructed Bayesian stable isotope mixing models using the R MixSIAR package (Stock *et al.*, 2018) to estimate the proportions of different prey sources (i.e., the three prey guilds including rice herbivores, tourist herbivores, and detritivores) in predators’ diet (Fig. Ac). The Bayesian framework allows for the incorporation of prior information on the diets of predators as well as various sources of uncertainties in the diet estimation (Moore and Semmens, 2008; Parnell *et al*., 2013). In the mixing models, individual farm-year combination and crop stage were included as fixed effects for predator isotope data; isotope data for the three prey guilds were pooled respectively to generate fixed source values because of their high mobility across farms (Mazzi and Dorn, 2012; Sun *et al.*, 2015). Isotope data at the seedling stage for the three study years were omitted from the mixing model analysis due to insufficient sample sizes (missing prey sources and low predator numbers) for reliable estimation of predators’ diet composition. To improve our model estimates, carbon and nitrogen concentration dependencies (the C and N contents of the isotope samples) as well as the residual/process errors were incorporated (Phillips and Koch, 2002; Stock and Semmens, 2016). Trophic discrimination factors (TDFs) were estimated from the diet-dependent discrimination equation proposed by Caut *et al.* (2009). We ran three Markov Chain Monte Carlo (MCMC) chains, each with 50,000 iterations and a burn-in number of 25,000, along with a non-informative Dirichlet prior (Stock *et al*., 2018). Chain convergence was assessed via Gelman-Rubin and Geweke diagnostics (Gelman and Rubin, 1992). Bayesian posterior median estimates of diet composition (for each year-farm-stage combination) were extracted for further analyses. Bayesian posterior means, SDs, medians, and 95% credible intervals are provided in Appendix B.

To examine how local abiotic and biotic factors may affect the pest consumption by GAPs (Fig. Ad), we fit weighted generalized linear mixed models (GLMMs) with a beta distribution and a logit link function using the R glmmTMB package (Brooks *et al.*, 2017), with year, farm type, crop stage, percent forest cover, and the relative abundance of rice herbivores as fixed effects, farm ID nested within pair ID as random effects, and the proportion of rice herbivores consumed in predators’ diet as the response (i.e., posterior medians from the Bayesian stable isotope mixing models). Weights were computed based on the number of diet estimates in each year. Model parameters were estimated using maximum likelihood, and their significance was analyzed via Wald chi-square test using the “Anova” function in the R car package (Fox and Weisberg, 2018). Tukey’s post-hoc tests (α = 0.05) were performed for the significant factors using the “cld” function in the R emmeans package (Length, 2018). The percent forest cover around each study farm was estimated from Google Earth images by manually delimiting the forested areas within a 1-km radius circular buffer surrounding the farm and computing the fraction of these areas in the buffer zone (Table SA). The 1-km radius was based on previous studies (Rusch et al., 2016; Karp *et al.*, 2018). Because spiders and ladybeetles may have different feeding behavior and preference, we also performed all the aforementioned analyses separately for each of the two predator groups. Additionally, we fit GLMMS with a negative binomial distribution and a log link function with the aforementioned model structure to examine the predator and rice herbivore abundance (i.e., the number of individuals in the sweep net samples) in the field. All analyses were conducted in R version 4.0.3 (R Core Team, 2021).

*2.5. Replication statement*

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| **Scale of inference** | **Scale at which the factor of interest is applied** | **Number of replicates at the appropriate scale** |
| Predator and prey stable isotope analysis and mixing model analysis for predators’ diet compositions | Predator, rice herbivore, tourist herbivore, and detritivore individuals collected at each rice stage in organic and conventional farms over three study years | 352 predator stable isotope samples (capsules)  828 prey stable isotope samples (capsules) |
| Patterns of pest consumption by predators in rice agro-ecosystems | Proportion of rice pests in predators’ diet at each rice stage in organic and conventional farms over three study years | 3 rice stages × 14 farms × 3 study years |

*2.6. Ethics statement*

Ethical approval was not required for this study.

**3. Results**

*3.1. Diet composition of predators in rice farms*

Across organic and conventional rice farms during 2017-2019, the proportion of rice herbivores in predators’ diet increased over the crop season from 21-47% at the tillering stage to 80-97% at the ripening stage; the proportion of detritivores in predators’ diet decreased from 35-61% at the tillering stage to <1% at the ripening stage; the proportion of tourist herbivores in predators’ diet also decreased from 13-20% at the tillering stage to 3-18% at the ripening stage (Fig. 1a; Appendix A: Table S2, Fig. S2).

Regarding individual predator groups, spiders and ladybeetles showed a marked difference in their diet composition over crop stages during 2017-2019. Across organic and conventional farms, spiders consumed a higher proportion of detritivores (31-55%) in their diet in the beginning of crop season (tillering stage) and substantially increased the consumption on rice herbivores to 78-95% in late crop season (ripening stage) (Fig. 1b; Appendix A: Table S2, Fig. S2). In contrast, ladybeetles in both organic and conventional farms consumed a low proportion of detritivores (≤ 8%) and a steadily high proportion of rice herbivores (≥ 80%) in their diet throughout the crop season (Fig. 1c; Appendix A: Table S2, Fig. S2). Tourist herbivores generally did not constitute an important prey source and contributed less than 33% to the diet of spiders and ladybeetles (Fig. 1b, 1c; Appendix A: Table S2, Fig. S2).

*3.2. Patterns of rice herbivore consumption by predators*

We further analyzed rice herbivore consumption by GAPs since these herbivores are the main pests of concern. The patterns of rice herbivore consumption by both predators in organic and conventional rice farms were generally similar across the three study years, suggesting consistency in GAPs’ feeding habits (Fig. 2). Interestingly, spiders and ladybeetles exhibited distinct within-season patterns of rice herbivore consumption. For spiders in organic and conventional farms, the proportion of rice herbivores in their diet increased toward later crop season, ranging from 17-48% (tillering) to 78-95% (ripening) (Fig. 2b; Appendix A: Table S2, Fig. S2), whereas for ladybeetles in organic and conventional farms, the proportion of rice herbivores in their diet remained relatively stable throughout the season, ranging from 80-93% (tillering) to 97-98% (ripening) (Fig. 2c; Appendix A: Table S2, Fig. S2).

*3.3. Factors associated with rice herbivore consumption by predators*

The proportion of rice herbivores in GAPs’ diet differed between organic and conventional farms for both predators (*χ*2 = 7.92, *P* = 0.01) and spiders(*χ*2 = 4.93, *P* = 0.03), but not ladybeetles (*χ*2 = 0.47, *P* = 0.49; Table 1). Specifically, predators in conventional farms consumed a higher proportion of rice herbivores in their diet compared to those in organic farms (Table 2). The proportion of rice herbivores in GAPs’ diet also differed among crop stages (both predators: *χ*2 = 249.84, *P* < 0.001; spiders: *χ*2 = 119.01, *P* < 0.001; ladybeetles: *χ*2 = 184.32, *P* < 0.001; Table 1). Specifically, GAPs consumed higher proportions of rice herbivores in their diet at the flowering and/or ripening stage compared to the tillering stage (Table 3).

The proportion of rice herbivores consumed in GAPs’ diet was not associated with the percent forest cover within a 1-km radius buffer surrounding the study farms (both predators: *χ*2 = 0.06, *P* = 0.80; spiders: *χ*2 = 0.12, *P* = 0.73; ladybeetles: *χ*2 = 0.34, *P* = 0.56; Table 1). Furthermore, the proportion of rice herbivores consumed was not associated with the relative abundance of rice herbivores in the field (both predators: *χ*2 = 0.56, *P* = 0.46; spiders: *χ*2 = 0.58, *P* = 0.45; ladybeetles: *χ*2 = 0.38, *P* = 0.54; Table 1).

Predator abundance (both spiders and ladybeetles) varied among years (*χ*2 = 64.2, *P* < 0.001) and crop stages (*χ*2 = 40.0, *P* < 0.001) but did not differ between farm types (*χ*2 = 0.006, *P* = 0.94) (Table SB–D). Rice herbivore abundance also varied among years (*χ*2 = 30.2, *P* < 0.001) but did not vary among crop stages (*χ*2 = 1.0, *P* = 0.60) or differ between farm types (*χ*2 = 0.63, *P* = 0.43) (Table SB–D). Percent forest cover did not affect predator (*χ*2 = 1.8, *P* = 0.18) or rice herbivore abundance (*χ*2 = 2.8, *P* = 0.10).

**4. Discussion**

Because the worldwide demand for environmentally friendly practices in agriculture has increased, we investigated the potential of GAPs (ubiquitous in nature) as biocontrol agents in rice agro-ecosystems. Specifically, we used stable isotopes to quantify the diet composition of GAPs in organic and conventional rice farms over the crop season for three consecutive years. Our main results include the following: 1) Across the three study years, the rice herbivore consumption by GAPs increased in both organic and conventional farms over the crop season, from 20-47% at the tillering stage to 80-97% at the ripening stage. The high proportion at the ripening stage indicates that GAPs could function as pest specialists during critical rice growth stages. Interestingly, rice herbivore consumption by spiders increased gradually toward the later crop season, whereas the consumption by ladybeetles remained stable throughout the season. 2) Our results revealed similar among-year patterns in rice herbivore consumption by GAPs in organic and conventional rice farms, suggesting a consistency in GAPs’ feeding habits and biocontrol value. 3) The proportion of rice herbivores in GAPs’ diets varied with farm type and crop stage (e.g., higher in conventional farms and at flowering/ripening stages). However, pest consumption by GAPs was not associated with percent forest cover or the relative abundance of rice herbivores in the field. We discuss in the following: 1) GAP’s role as pest specialists at late crop stages, 2) consistency in pest consumption by GAPs patterns over years, 3) factors associated with pest consumption by GAPs, and 4) the potential caveats of this study (e.g., pest suppression and intraguild predation). We finish by highlighting the implications of our results for agricultural management.

*4.1. Generalist predators function as pest specialists at late crop stages*

While biocontrol, a farming practice with a long history, offers a promising solution for sustainable agriculture, the use of GAPs as biocontrol agents remains a concern because GAPs may switch diets between pests and alternative prey (Albajes and Alomar, 1999; Prasad and Snyder, 2006; Roubinet *et al.*, 2018). This study addressed this concern and revealed a consistency in high pest consumption by GAPs at late crop stages over years. The results provide not only strong support for using GAPs in sustainable pest management, but also a novel aspect in biocontrol—generalist predators may function as guild-level specialist predators of pests during the late crop season. Specifically, across the three study years, GAPs in both organic and conventional farms consumed an increasing proportion of rice herbivores over the crop season, reaching 80-97% in predators’ diet at the ripening stage, whereas the proportions of alternative prey (detritivores and tourist herbivores) gradually decreased below 18% at the ripening stage (Fig. 1; Appendix A: Table S2, Fig. S2). These changes in dietary proportions may result from variations in the relative prey abundance in the field over the growing season (Fig. SX). Moreover, the increase in rice herbivore consumption over time suggests that the biocontrol potential of predators increases toward late crop stages and peaks at the critical stage of crop production.

While GAPs consumed a high proportion of pests at late crop stages, the two major predator groups in our study system, spiders and ladybeetles, exhibited distinct dietary patterns over the crop season. Specifically, pest consumption by spiders increased substantially, but pest consumption by ladybeetles remained stable over the season (Fig. 2b vs. 2c). This may be because different foraging modes—sit-and-wait (spiders) or actively hunting (ladybeetles)—can lead to different prey capture and thus diet composition (Nyffeler, 1999; Klecka and Boukal, 2013). For example, the dominant spider genus in our study, *Tetragnatha* (long-jawed orb-weavers), consists of sit-and-wait predators, and their diet composition generally reflects prey availability (Nyffeler, 1999). In contrast, ladybeetles are actively hunting predators and may preferentially feed on rice herbivores, resulting in stable pest consumption over time. Because predator foraging modes shape predator-prey-plant interactions (Schmitz, 2008), future research focusing on different assemblages of sit-and-wait vs. actively hunting predators (e.g., web-building vs. hunting spiders) in field conditions will help better understand within-predator-guild variation in pest consumption and biocontrol efficacy over crop season.

*4.2. Generalists exhibit consistent pest consumption patterns over years*

Ideal biocontrol agents provide a consistent, predictable effect on pests under various environmental conditions. Accordingly, GAPs in this study showed consistent pest consumption across years, despite variable abiotic and biotic conditions. Regarding the abiotic factors, the daily mean temperature, particularly from April to June, varied substantially among years (Appendix A: Fig. S3). The daily precipitation also fluctuated over the three study years, with multiple high precipitation events in 2017, overall low precipitation in 2018, and relatively uniform precipitation in 2019 (Appendix A: Fig. S3). Regarding the biotic factors, the abundance of different prey sources (Table SE) as well as the composition of rice herbivores at the flowering and ripening stages differed substantially among the three years, in particular the two most dominant groups: leafhoppers (Cicadellidae/*Nephotettix*) and planthoppers (Delphacidae/*Nilaparvata*) (Appendix A: Table S3). Although these factors varied substantially over the three study years, pest consumption by GAPs generally remained stable, suggesting that GAPs can be predictable biocontrol agents in rice fields (but see Eitzinger *et al.*, 2021).

*4.3. Factors associated with pest consumption by predators*

The proportion of rice pests in GAPs’ diets differed between farm types and among crop stages but was not associated with the percent forest cover surrounding the farms or the relative abundance of rice herbivores in the field. Overall, GAPs in conventional farms consumed a higher proportion of rice pests in their diet compared to those in organic farms. There are two explanations for this: 1) Organic farming may promote arthropod diversity and therefore distract predators from feeding on target pests (Bengtsson *et al.*, 2005; Birkhofer *et al.*, 2008; Lichtenberg *et al.*, 2017). 2) Pest densities may be higher in conventional farms (Porcel *et al.*, 2018), leading to higher predator-prey encounter rates and thus pest consumption by GAPs. In fact, although not statistically significant, rice herbivore abundance appeared to be higher in conventional farms (Table SC). Regardless of the potential mechanisms, our results indicate an important but overlooked biocontrol value of GAPs in conventional farming systems.

Besides farming practices, crop stages also affected pest consumption. Overall, pest consumption by GAPs increased from early (tillering) to late (ripening) stages, consistent with previous studies where predators consumed more pests in the late crop season (Roubinet *et al.*, 2017; Hsu *et al.*, 2021). Therefore, farming practitioners may avoid practices that harm predators (e.g., chemical applications) during this period to maintain healthy predator populations and the associated ecosystem services.Interestingly, rice herbivore abundance did not increase significantly over the crop season (Table SD). Yet, GAPs still consumed increasing proportions of rice pests at late crop stages, suggesting that prey preference may play a role in their feeding behavior.

Complex habitat structure (e.g., surrounding vegetation) has been suggested to promote predator abundance and diversity (Langellotto and Denno, 2004; Diehl *et al.*, 2013), but such higher complexity did not affect predators’ diet composition in our study. This might be because the prey species in our study system were mostly associated with rice plants but not the surrounding vegetation, consistent with a meta-analysis where habitat complexity had no effect on crop herbivore densities (Langellotto and Denno, 2004). Furthermore, although the diet composition of generalist predators may correlate with prey availability in the field (Wise *et al.*, 2006; Hsu *et al.*, 2021), our model results suggest no such correlation between the relative abundance of rice herbivores and pest consumption by GAPs. An explanation is that the relative abundance of rice herbivores was highly correlated with crop stage, a significant factor associated with various covariates (e.g., rice plant height) and likely explaining most variations in pest consumption by GAPs. We encourage further experiments, both observational and manipulative, to clarify the link between prey availability and generalist predators’ diet composition in the field.

*4.4. Potential caveats of this study*

Our study demonstrates high pest consumption by GAPs in rice fields over three years and examines the factors influencing GAPs’ diet composition. While our study provides evidence for GAPs’ biocontrol potential, some caveats may exist. First, high pest consumption in GAPs’ diets does not necessarily imply a strong suppression of pest populations in the field, since pest population dynamics depend not only on the per capita effect of predators but also predator density and diversity (Letourneau *et al.*, 2009; Rusch *et al.*, 2016). To unveil the connection between per capita pest consumption and overall pest dynamics, future work may complement stable isotope analysis with field observations of predator and pest populations as well as crop production to reveal the overall effects of GAPs on pest control and crop performance.

Another factor that can potentially affect predator-pest interactions and GAPs’ biocontrol effectiveness is intraguild predation.

For example, XXX

while intraguild predation potentially influences the pest control by GAPs (Straub *et al.*, 2008; Michalko *et al.*, 2019),

We did not attempt to quantify intraguild predation in our diet composition analysis because of the limitation of stable isotope mixing models (including predator isotope data as both prey source and consumer is not recommended) (Hsu *et al.*, 2021).

However, this may not be a major concern in our study because rice plants grow in dense clumps (especially at the late crop season; Fig. Ab) and form a complex structure that could substantially relax intraguild predation pressure (Finke and Denno, 2006; Janssen *et al.*, 2007).

Moreover, the isotope signatures of predator individuals lie well within the prey source polygon (Fig. S1), suggesting that intraguild predation, if not absent, might be relatively minor.

Regardless, we caution that our diet estimates of predators (without predator-predator interference) might not apply to systems where intraguild predation prevails.

**5. Conclusions**

While biocontrol has been recognized as a valuable approach to sustainable agriculture, whether generalist predators can serve as effective biocontrol agents in pest management remains unclear. Our study helps solve this long-standing puzzle by using stable isotope analysis to quantify the diet composition of generalist arthropod predators (spiders and ladybeetles) over the rice growing season and by identifying the underlying mechanisms for enemy-pest interactions in rice farms for three consecutive years. The results show a high proportion of rice pests in predators’ diets in both organic and conventional rice farms (e.g., 80-97% at the ripening stage), suggesting that these generalist predators function as “pest specialists” at late crop stages when rice plants are fruiting. The high pest consumption remained consistent across years regardless of fluctuations in abiotic and biotic conditions, demonstrating that generalist predators may produce a stable, predictable top-down effect on pests. Overall, our study lends support to applying generalist predators as biocontrol agents in both organic and conventional rice farms. As sustainable agriculture has become more important than ever in human history, incorporating the ubiquitous generalist predators into pest management and maintaining healthy populations of these predators will likely open a promising avenue towards this goal.

**Appendix A and B. Supporting information**

Supplementary information associated with this article can be found in the online version at doi:xxx.

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**Tables**

**Table 1.** GLMM results for the effects of various farm factors on pest consumption by spiders, ladybeetles, and both predators.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Factor | *d.f.* | *χ*2 | *P* |
| Both predators | Year | 2 | 8.00 | 0.02 |
|  | Farm type | 1 | 7.29 | 0.01 |
|  | Crop stage | 2 | 249.84 | < 0.001 |
|  | Percent forest cover | 1 | 0.06 | 0.80 |
|  | Relative abundance of rice herbivores | 1 | 0.56 | 0.46 |
| Spiders | Year | 2 | 9.30 | 0.01 |
|  | Farm type | 1 | 4.93 | 0.03 |
|  | Crop stage | 2 | 119.01 | < 0.001 |
|  | Percent forest cover | 1 | 0.12 | 0.73 |
|  | Relative abundance of rice herbivores | 1 | 0.58 | 0.45 |
| Ladybeetles | Year | 2 | 17.29 | < 0.001 |
|  | Farm type | 1 | 0.47 | 0.49 |
|  | Crop stage | 2 | 184.32 | < 0.001 |
|  | Percent forest cover | 1 | 0.34 | 0.56 |
|  | Relative abundance of rice herbivores | 1 | 0.38 | 0.54 |

**Table 2.** Tukey’s post-hoc tests comparing the proportion of rice herbivores consumed in the diet of predators in organic and conventional rice farms. Different superscript letters indicate significant differences in the estimated marginal means (EMMs) of the posterior medians from Bayesian stable isotope mixing models (α = 0.05).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Farm type | EMMs (± SE) | Lower 2.5% | Upper 2.5% |
| Both predators | Organic | 0.61a (± 0.08) | 0.45 | 0.76 |
|  | Conventional | 0.81b (± 0.05) | 0.69 | 0.90 |
| Spiders | Organic | 0.55a (± 0.10) | 0.35 | 0.73 |
|  | Conventional | 0.79b (± 0.07) | 0.63 | 0.90 |
| Ladybeetles | Organic | 0.95a (± 0.01) | 0.93 | 0.96 |
|  | Conventional | 0.95a (± 0.01) | 0.94 | 0.96 |

**Table 3.** Tukey’s post-hoc tests comparing the proportion of rice herbivores consumed in the diet of predators at three crop stages (tillering, flowering, and ripening stages). Different superscript letters indicate significant differences in the estimated marginal means (EMMs) of the posterior medians from Bayesian stable isotope mixing models (α = 0.05).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Crop stage | EMMs (± SE) | Lower 2.5% | Upper 2.5% |
| Both predators | Tillering | 0.24a (± 0.06) | 0.14 | 0.36 |
|  | Flowering | 0.85b (± 0.04) | 0.76 | 0.91 |
|  | Ripening | 0.91c (± 0.03) | 0.85 | 0.95 |
| Spiders | Tillering | 0.27a (± 0.07) | 0.16 | 0.43 |
|  | Flowering | 0.81b (± 0.05) | 0.69 | 0.89 |
|  | Ripening | 0.86b (± 0.04) | 0.75 | 0.93 |
| Ladybeetles | Tillering | 0.92a (± 0.01) | 0.89 | 0.93 |
|  | Flowering | 0.92a (± 0.01) | 0.90 | 0.93 |
|  | Ripening | 0.98b (± 0.01) | 0.98 | 0.99 |

**Figures**

**Figure 1.** The proportions (mean ± SE) of prey sources (rice herbivores, tourist herbivores, and detritivores) consumed in the diet of (a) both predators, (b) spiders, and (c) ladybeetles in organic and conventional rice farms over crop stages. The proportions were computed from the Bayesian posterior medians of diet estimates in replicate farms over the three study years.

**Figure 2.** The proportion (mean ± SE) of rice herbivores consumed in the diet of (a) both predators, (b) spiders, and (c) ladybeetles in organic and conventional rice farms over crop stages in the three study years. The proportions were computed from the Bayesian posterior medians of diet estimates in replicate farms.

**Figure 3.** The relative abundance of prey sources in organic and conventional rice farms over crop stages during the three study years. The relative abundance was determined from the sweep-net samples pooled across replicate farms.

**Figure A.** The sampling design and analyses in the study. (a) A map of the paired organic and conventional rice farms in the three study years (three farm pairs in 2017 and seven farm pairs in 2018 and 2019). The red rectangle in the inset map denotes the area in central Taiwan where the farms were located. (b) In each farm, arthropods were sampled at four major rice stages (seedling, tillering, flowering, and ripening stage) via the sweep net method. (c) The field arthropod samples were assigned to three prey guilds (rice herbivore, tourist herbivore, and detritivore) and one predator guild, and the proportions of these three prey sources consumed in predators’ diets were quantified via stable isotope analysis. (d) The proportion of rice herbivores consumed in predators’ diets was extracted from (c) to examine how pest consumption by predators varied with local farm factors such as farm type, crop stage, percent forest cover, and the relative abundance of rice herbivores in the field.

**Figure 1.**

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**Figure 2.**

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**Figure 3.**

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**Figure A.**

